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Research Article

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A Study of Tumour Infiltrating Lymphocytes (Tils) In Breast Carcinoma and their Immuno-Histochemical Profile

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Abstract

Objective: *Tumor-infiltrating lymphocytes (TILs) play an important role in mediating immune response against cancer cells and are associated with improved clinical outcomes. The present study was conducted to demonstrate the relative densities of T lymphocytes, CD4+ cells, CD8+ cells, and B-lymphocytes in a series of breast carcinoma.*

Material and method: *Thirty cases of primary breast carcinoma were examine for the histological parameters including histological type, histological grade and lymph node metastases and pattern of inflammatory reaction. Further, immunohistochemical expression in cases with lymphocytic infiltrate was studied in order to classify various lymphocyte subsets.*

Results: *T-cells (CD3+) were present in 96.6% cases of breast cancer, majority of which were CD8+ cells in 90% cases and CD4+ cells in 36.6% cases. B-cells were present in 83.3% cases. Also, insignificant correlation was found between TILs and subtypes with age, menopausal status, histological grades, ER, PR and HER2/neu status and tumor size. No significant correlation was found between CD4, CD8 and CD20 with lymph nodes stage in our study. However, significant correlation was found between CD3 with lymph nodes stage.*

Conclusion: *T lymphocytes and their subsets (especially CD8+ cells) predominated over B lymphocytes quantitatively and qualitatively. They seem to promote neoplastic progression rather than acting as a protective immune response against cancer.*

Keywords: Breast Cancer, Tumor-Infiltrating Lymphocytes, Prognostic Factor, Immune Response, Immunohistochemistry

Introduction

Breast carcinoma is one of the leading causes of cancer death in women worldwide [1]. Tumor infiltrating lymphocytes (TIL) have predicted good outcome in many tumors including colon, ovarian, lung and breast cancer. In breast cancer, the presence of tumor-infiltrating lymphocytes (TILs) in the tu¬mor or peritumoral site has been recognize as an important biomarker of antitu¬mor immune response [2]. Despite the heterogene¬ity of TILs and the absence of a standardized methodology of evaluating TILs, it was suggest that the presence of TILs prior to treatment is associated with good prognosis. In a recent study, it was observed that most TILs are T cells, accounting for 75% of total TILs, out of which, CD8+ T cells constituted the largest group and an associated with overall favourable clinical outcomes and longer survival rates [3]. Keeping in view of TIL in breast cancers, new therapies have been introduced that reactivate anticancer immune responses to cancer [4]. However, on the other hand, it also reported that TILs play a role in the spread of carcinoma, both locally and distant metastasis with two proposed hypotheses: One is that lymphocytic infiltrate merely reflects nonspecific inflammatory reaction resulting from tumor-derived chemokines and cytokines, and the other is that they represent a specific immunologic reaction [5]. The present study was undertaken with an aim to demonstrate the relative densities of T lymphocytes, CD4+ cells, CD8+ cells, and B-lymphocytes in a series of breast carcinoma. The relationship between the density of each cell immunophenotype and the clinicopathologic factors of prognostic significance were also assessed [6, 7].

Material and Method

A total of thirty cases of radical or modified radical mastectomy from cases of primary breast carcinoma constituted the study group. Patients with breast cancer other than primary adenocarcinoma such as lymphoma, sarcoma, stromal tumor, metastases were excluded. Specimens were examined grossly for tumor size, consistency, margin, and cut surface along with axillary lymph node status. Paraffin blocks were prepared from representative areas and hematoxylin and eosin (H & E) stained sections were retrieved, as per standard procedure. Cases were examined for the histological parameters including histological type, histological grade (Modified Bloom Richardson grading system and NPI) and lymph node metastases. Estrogen and progesterone receptor status was assessed by Quick score taking into account both percentage of tumor cells and intensity of staining. Pattern of inflammatory reaction (lymphocytes, neutrophils, plasma cells and macrophages) in H & E stained sections was also noted. Further immunohistochemical expression in cases with lymphocytic infiltrate was studied in order to classify various lymphocyte subsets i.e., B and T cells, CD4+ and CD8+ T cells respectively.

Scoring of Lymphocytes Infiltrate

Areas of highest lymphoid density were selected and necrotic areas were avoided. The density of total lymphocytic infiltrate in H & E stained section and the density of each cell immunophenotype in the immunostained slides was semiquantitatively graded into Absent, minimal (<10 lymphocytes / high power field 40x), moderate (lymphocytes easily identified but no large aggregates) and extensive (large aggregates of lymphocytes in more than 50% of the tumor) [8, 9]. For statistical purposes, minimal and moderate grades were grouped as intermediate. Immunohistochemical profile for TILs was studied, after staining with CD3, CD20, CD4 and CD8 and their relative proportion assessed.

Statistical Analysis

Data was analysed statistically by using Chi-square test. Chi-square and Spearman's correlation test was used to assess the relationship between original data.

Results

In the study, age of the patients ranged from 24-70 years with a mean age of 53±10 years. Out of which, maximum number of cases 12/30 (40%) belonged to age group of 51-60 years. Eight (26.7%) out of thirty cases were premenopausal.

CD3+ cells were present in 29 (96.6%) cases of breast cancer followed by CD8+ cells in 27 (90%) cases, B-cells in 25 (83.3%) and CD4+ cells in 11 (36.6%) cases. On statistical analysis, expression of tumor infiltrating lymphocytes as tumor inflammation was significant ($p<0.05$).

Table 1: Distribution and Comparison of Tils and Their Subsets in Breast Carcinoma

Lymphocytes	Absent	PRESENT				(P value) Chi- square
		Intermediate	Extensive	Total	Percentage %	
T-CELL(CD3 +)	1	19	10	29	96.6	0.000
B-CELL	5	23	2	25	83.3	0.000
CD4+ CELLS	19	10	1	11	36.6	0.000
CD8+CELLS	3	25	2	27	90	0.000

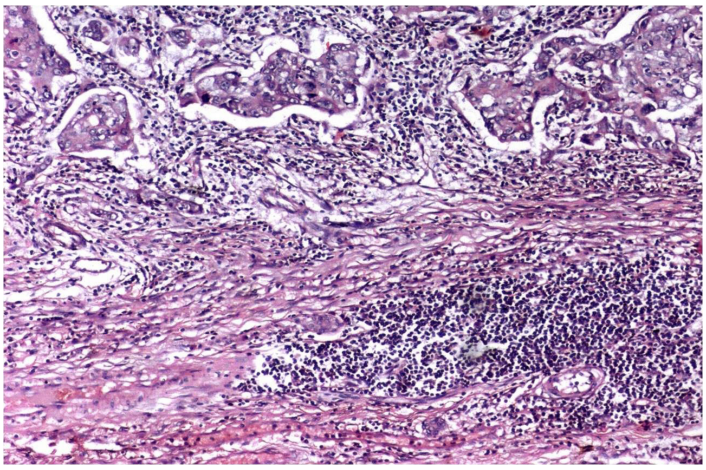

Figure 1: Infiltrating ductal carcinoma of breast (NOS) with peripheral lymphocytic infiltration. (H&E, 100x)

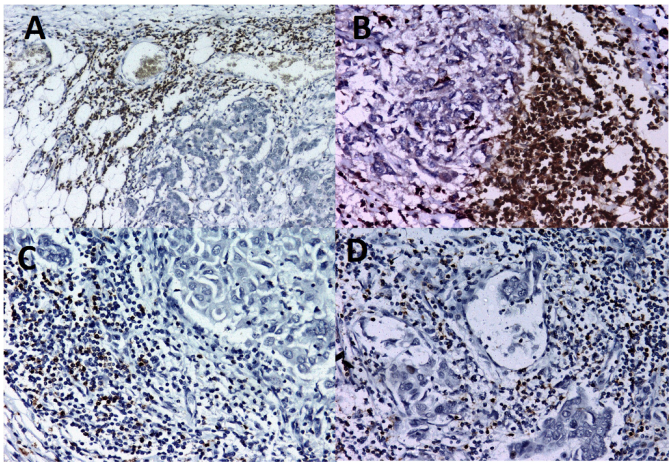

Figure 2: Infiltrating ductal carcinoma of breast (NOS) infiltration with A) with peripheral lymphocytic infiltration showing CD3+ expression. (IHC, 100x), B) intratumoral lymphocytic infiltration showing CD3+ expression. (IHC, 200x), C) peripheral lymphocytic infiltration showing CD4+ expression. (IHC, 200x), and D) intratumoral lymphocytic infiltration showing CD4+ expression. (IHC, 200x**)**

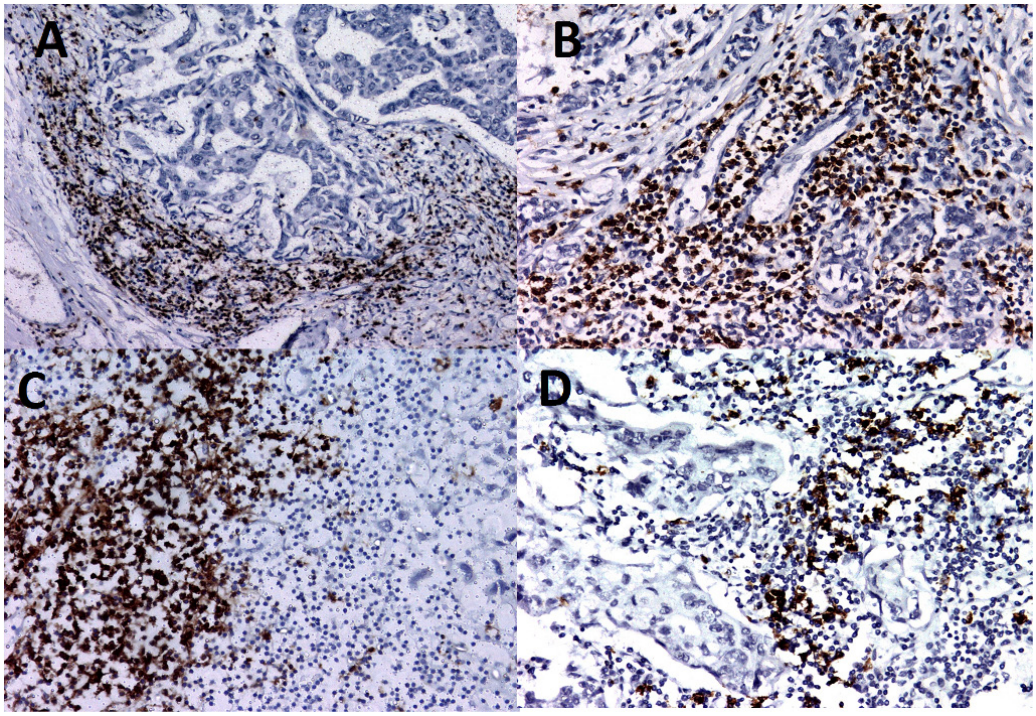

Figure 3: Infiltrating ductal carcinoma of breast (NOS) infiltration with A) with peripheral lymphocytic infiltration showing CD8+ expression. (IHC, 100x), B) intratumoral lymphocytic showing CD8+ expression (IHC, 200x), C) peripheral lymphocytic infiltration showing CD20 expression. (IHC, 200x), and D) intratumoral lymphocytic infiltration showing CD20 expression. (IHC, 200x)

In lymph node stage I (n=11) and stage II (n=14), the maximum number of cases showed positive expression for CD3 followed by CD8 and CD20 while, in lymph node stage III (n=5), CD3 followed by CD20 and CD8. Maximum number of cases for CD4 belonged to absent type in all the lymph node stage I, II and III. No significant correlation was found between CD4, CD8 and CD20 with lymph nodes stage in our study. However, significant correlation was found between CD3 with lymph nodes stage. Table 2 Insignificant correlation was found between ratio of CD4/CD8 with tumor histological grade, lymph node stage, tumor size, NPI score and hormonal status (ER, PR and Her2/neu) group in our study. Table 3

Table 2: Relationship between the Density of Tumour Infiltrating Lymphocytes and Lymph Node Status of Breast Carcinoma

Lymph node staging	CD3 (%)			CD4 (%)			CD8 (%)			CD20 (%)		
	A	I	E	A	I	E	A	I	E	A	I	E
I (n=11)	-	10 (52)	1 (10)	9 (40)	2 (29)	-	1 (34)	10 (40)	-	3 (60)	8 (35)	-
II (n=14)	-	8 (42)	6 (60)	10 (46)	4 (57)	-	-	13 (52)	1 (50)	1 (20)	11 (48)	2 (100)
III (n=5)	1 (100)	1(8)	3 (30)	3 (14)	1 (14)	1 (100)	2 (66)	2 (8)	1 (50)	1(20)	4 (17)	-
TOTAL	1	19	10	22	7	1	3	25	2	5	23	2
P value (Chi-square)	0.024			0.235			0.053			0.425		
Spearman's correlation	r=0.311 p=0.094			r=0.239 p=0.203			r=-0.006 p=0.976			r=0.168 p=0.374		

Table 3: Correlation between Histological Grade, Lymph Node Status, Er, Pr, Her2neu Status, Npi with Cd4/Cd8 Ratio

HISTOLOGICAL PARAMETERS		CD4/CD8 Absent	CD4/CD8 Intermediate	CD4/CD8 Extensive	P- value (chi-square)
Histological grade	I (n=10)	6/1	4/9	0/0	0.244
	II (n=15)	9/2	6/12	0/1	
	III (n=5)	3/0	1/4	1/1	
Lymph node stage	I (n=11)	9/1	2/10	0/0	0.235
	II (n=14)	10/0	4/13	0/1	
	III (n=5)	3/2	1/2	1/1	
Tumour size	<2cm (n=3)	1/0	2/3	0/0	0.708
	2-5 cm (n=25)	17/3	7/20	½	
	>5 cm (n=2)	1/0	1/2	0/0	
NPI	Good (n=6)	3/0	3/6	0/0	0.265
	Moderate (n=18)	13/2	5/15	0/1	
	Poor (n=6)	3/1	2/4	1/1	
ER status	Positive (n=19)	12/2	7/16	0/1	0.383
	Negative (n=11)	7/1	3/10	1/0	
PR status	Positive (n=18)	10/2	8/16	0/0	0.166
	Negative (n=12)	9/1	2/9	1/2	
Her2neu status	Positive (n=14)	9/0	4/12	1/2	0.516
	Negative (n=16)	10/2	6/14	0/0	

Discussion

The exact role of the tumor-infiltrating lymphocytes with respect to breast cancer and its outcome has not been clearly defined. It has been previously suggested that the presence of TILs is an indication of favourable prognosis. Conversely, it was also claimed that T lymphocytes play a role in the local and metastatic spread of carcinoma to lymph nodes. The relationship of B lymphocytes in breast cancer has been investigated as a prognostic factor in few studies [7].

In our study T-cells (CD3+) were present in 29 cases (96.6%) of breast cancer while B-cells in 25 cases (83.3%). Also, CD8+ cells were present in 27 cases (90%) & CD4+ cells in 11 cases (36.6%). Helal et al7 observed that the majority of TILs were T lymphocytes

which were present in all 39 carcinomas with TIL (81.3% of all carcinomas). On the contrary, B lymphocytes were noted in 24 of the 39 tumors with TIL (50% of all carcinomas). CD4+ cells were a constant component of T lymphocytes in all 39 tumors while CD8+ cells were noted in 27 of the 39 tumors. So, they found that CD4 expression was more than CD8. The present study was not in agreement with Helal study in view of distribution of TIL's and their subsets in cases of B cell, CD4 and CD8 cells whereas T cells had similar expression in both studies [7].

In the present study, it was found that maximum number of cases in all the age groups belonged to positive for CD3, CD8 and CD20 while CD4 maximum cases belonged to 'Absent' type. There was no significant correlation between TILs and subtypes with age. Our study was concordance with Rathore and other literature refuted our findings for T cell, CD4, and CD8 expression while for CD20 it was in concordance with Helal [7, 10]. In study of Rathore (n=150), CD3+, CD4+ and CD8+ TIL counts were not significantly associated with age [11]. On the other hand in the study of Helal et al7, the density of TILs, T lymphocytes, CD4+ and CD8+ cells showed a significant direct association with patient age (P = 0.003, P =0.004, P = 0.001, and P = 0.01, respectively). Maccheti et al11 (n=23) observed that CD4 expression was significantly associated with age.

Maximum number of cases in premenopausal group (n=8) showed positive expression for CD3 (8/8) and CD8 (7/8) whereas in postmenopausal group (n=22), maximum cases were CD3 (21/22), CD8 (20/22) and CD20 (18/22) positive. No significant correlation was found between TILs and subtypes with menopausal status in our study. In study of Rathore (n=150), CD3+, CD4+ and CD8+ TIL counts were not significantly associated menopausal status. Maccheti et al11 (n=23) observed that CD4 expression was significantly associated with menopausal status [10].

Fifteen (50%) out of thirty cases belonged to grade II followed by ten cases of grade I (33.7%). In histological grade I, grade II and grade III, maximum number of cases revealed higher positive density for CD3, CD8, and CD20 while CD4 was more negative as compared to positive group. No significant correlation was found between TILs and subtypes with histological grades in our study. Helal et al7 (n=48) observed that poorly differentiated carcinomas (grade 3) had significantly higher density of TILs, T lymphocytes, and CD4+ cells than better differentiated tumors (grade 2) (P=0.003, P=0.004, and P=0.001, respectively). However, CD20 and CD8 expression was insignificant. But in their study, no cases were in histological grade I. Maximum number of cases were in (36/48) in grade III followed by 12 cases in grade II. Rathore also found significant association of CD3+, CD4+ and CD8+ TILs count with higher tumor grade. CD8 expression was seen different grade as follow: grade I (72%), grade II (80%) and grade III (81%) in study by Mahmood which was significant [11, 12]. Matkowski also observed insignificant association of CD4 and CD8 with tumor grade [13]. Aaltomaa observed insignificant association with tumor grade [14]. Our study was in concordance with the findings of Aaltoma and not in concordance with the other studies for CD3 and for CD4 in concordance with Matkowski while it was not in agreement with other studies. This study was in concordance with the studies while not in concordance with other studies for CD8 and for CD20 it is in concordance with studies [7, 10, 12-14].

The present study revealed that maximum number of positive and negative cases of ER, PR and HER-2neu showed expression for CD3 followed by CD8, and CD20. While for CD4, maximum number cases belonged to absent type with no significant correlation was found between TILs and subtypes with ER, PR and Her2/neu status. In study of Mahmood ER positive and negative cases showed CD8 expression 79% and 81% respectively [12]. PR positive and negative cases showed CD8 expression 78% and 80% respectively. Her2/neu positive and negative cases showed CD8 expression 82% and 79% respectively. Significant correlation of CD8 was found between ER and PR status. No significant association of CD4 and CD8 with ER and PR status was found by Matkowski [13]. CD4 expression was significantly was associated insignificantly with hormonal status in study of Maccheti Our study was concordant with the other studies for CD4 with respect to ER, PR and Her2/neu status while it was concordant with Matkowski for CD8 and disconcordant with other studies with respect to ER, PR and Her2neu status [10-13].

In lymph node stage I, II and III, maximum number of cases showed positive expression for CD3 followed by CD8, and CD20. While for CD4, maximum number cases belonged to absent type. No significant correlation was found between CD4, CD8 and CD20 with lymph nodes stage in our study. However, significant correlation was found between CD3 with lymph nodes stage. CD4 expression was significantly associated with lymph node status in study by Maccheti et al [11]. In study by Aaltomaa et al, lymphocytes expression was significantly associated with lymph node status [14]. Significant association of CD4 and CD8 with lymph node status was found by Matkowski et al [13]. Rathore also found significant association of CD3+, CD4+ and CD8+ TIL counts with lymph node status. Our study was in concordance with the findings of Aaltoma and Rathore for CD3 and for CD4, CD8 and CD20 not in agreement with other studies with respect to lymph node status [10-13].

In the present study, CD8+ cells were more prevalent than CD4+ cells. Plausible these data agree with some authors reports; the reverse was reported by others. The most for these contradictory results are variations in patients' or tumor selection, the use of different techniques in tissue processing (flow cytometry vs. immunohistochemistry), and the use of different methods in interpretation of expression of immunomarkers (quantitative vs. semi-quantitative). In comparison to T lymphocytes and their subsets, B lymphocytes had no significant association with any of the clinicopathologic parameters. Such relationship was investigated by a few reports that yielded the same results.

Conclusion

Hence, to conclude, a critical analysis of available literature, present study and other similar studies conducted so far, have although established an important role of immunophenotyping of lymphocytic subsets in progression of carcinoma breast, larger cross-sectional studies are needed to assess their prognostic significance as an adjunct to other clinicopathologic factors as well as an independent parameter.

Conflicts of interest:

The authors declare no conflict of interest.

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